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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/501,777
Filing Date: July 19, 2004
Appellant(s): BIRCH ET AL.

B.J. Sadoff
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 7/12/2010 appealing from the Office action mailed 9/28/2009.

(1) Real Party in Interest

The examiner has no comment on the statement, or lack of statement, identifying by name the real party in interest in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The following is a list of claims that are rejected and pending in the application:

claims 32 - 41 are pending.

(4) Status of Amendments After Final

The examiner has no comment on the appellant's statement of the status of amendments after final rejection contained in the brief.

(5) Summary of Claimed Subject Matter

The examiner has no comment on the summary of claimed subject matter contained in the brief.

(6) Grounds of Rejection to be Reviewed on Appeal

The examiner has no comment on the appellant's statement of the grounds of rejection to be reviewed on appeal. Every ground of rejection set forth in the Office action from which the appeal is taken (as modified by any advisory actions) is being maintained by the examiner except for the grounds of rejection (if any) listed under the subheading "WITHDRAWN REJECTIONS." New grounds of rejection (if any) are provided under the subheading "NEW GROUNDS OF REJECTION."

(7) Claims Appendix

The examiner has no comment on the copy of the appealed claims contained in the Appendix to the appellant's brief.

(8) Evidence Relied Upon

5,891,693	Bebbington et al.	4-1999
5,238,821	Barsomian et al	8-1993
6,395,484	Brandt et al	5-2002
6,096,555	Hermentin et al	8 - 2000
WO 87/04462 A1	Wilson et al	7 - 1987

Schneider, M. et al., "The importance of ammonia in mammalian cell culture", J. Biotech., 1996, Vol. 46: pp. 161 - 185.

Gawlitzek, M. et al., "Ammonium alters N-glycan structures of recombinant TNFR-IgG: Degradative versus biosynthetic mechanisms", Biotech. and Bioeng., Jun 2000, Vol. 68: pp. 637 - 646.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 32-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wilson et al. (WO 87/04462 A1) or Bebbington et al (U.S. Patent No. 5,891,693) as evidenced by Barsomian et al (U.S. Patent No, 5,238,821) in view of Brandt et al (U.S. Patent No. 6,395,484), Schneider et al. (Journal of Biotechnology, 1996), Gawlitzek et al. (Biotech. and Bioeng., Jun 2000) and Hermentin et al. (U.S. Patent 6,096,555).

Wilson et al. teach the use of a recombinant DNA sequence, using glutamine synthetase as a selectable marker in glutamine auxotrophic cells to express tissue plasminogen activator (tPA; see page 8, lines 4-33; pages 9-11; Figure 3). Barsomian et al disclose that tPA is a sialylated glycoprotein (see column 2, lines 49-52, for example).

Bebbington et al teach mouse and rat lymphoid cell lines that can be transformed to glutamine independence by incorporating a gene encoding glutamine synthetase (GS) so that the cells can grow in glutamine-free medium. Bebbington et al teach that the cell preferably contains a gene coding for a heterologous protein in which the gene is encoded on a separate vector (see column 2, lines 8-20, 25-30 and 44-51, for example), which meets the limitation of exogenous DNA sequences being located on more than one DNA construct. Bebbington et al teach

examples of heterologous proteins as human growth hormone, tPA, or tissue inhibitor of metalloproteinase (see column 2, lines 31-36, for example). Bebbington et al teach that the expression of the heterologous protein is substantially increased by selection for GS gene amplification (see column 2, lines 52-55, for example), which meets the limitation of GS as a selection marker. As stated above, Barsomian et al disclose that tPA is a sialylated glycoprotein (see column 2, lines 49-52, for example).

Bebbington et al do not teach a human glutamine-auxotrophic cell. Bebbington et al do not teach an exogenous sequence encoding a sialylated protein that further comprises a selectable marker such as claimed. Bebbington et al do not teach growing the cells in serum-free medium.

Brandt et al teach human cells (HT1080) for the preparation of human proteins in order to produce human proteins in economical yields in a form that is suitable for production of a pharmaceutical preparation (see column 1, lines 12 - 17, for example) which meets the limitation of glutamine-auxotrophic human cell line or an immortalized fibrosarcoma HT1080 cell line. Brandt et al teach the advantage of using serum free culture medium for culture of human cells because purification of proteins from serum free culture is substantially easier and has no danger of contamination with animal pathogens (see column 2, lines 22-34, for example) which meets the limitation of a human cell growing in a serum free culture. Brandt et al teach that it is advantageous to use a human cell line that synthesizes a desired protein with a glycosylation pattern, especially a sialic acid profile comparable to that of the naturally occurring target protein (see column 3, lines (19 - 30, for example). Brandt et al teach an HT1080 cell line (see column

8, lines 24-50, for example), which meets the limitation of a glutamine-auxotrophic human cell line or an immortalized fibrosarcoma HT1080 cell line (instant claims 38 - 39).

Brandt et al teach that the cells are cultured in serum-free medium and in suspension (see column 6, lines 25-42, for example). Bebbington et al teach that cells can be transformed to glutamine independence by incorporating a gene encoding GS so that the cells can grow in glutamine-free medium. Therefore, the cells made obvious by Bebbington et al in view of Brandt et al would be capable of growing in suspension in serum-free and glutamine-free medium (instant claim 36).

Brandt et al teach that the cells were cultured to produce the target protein, which was then recovered and quantified from the cell supernatant (see column 6, lines 44-63, for example), which meets the limitation of a process for the production of a sialylated protein in a glutamine-auxotrophic human cell in a serum free medium and recovery of the protein (instant claim 33).

Brandt et al also teach that a negative or positive selection marker or amplification gene can be included and can be DHFR, adenosine deaminase, ornithine decarboxylase or a thymidine kinase gene (see column 4, lines 56-65, for example), which meets the limitation of claims 34 and 35.

None of Wilson, Bebbington, Brandt or Barsomian et al specifically teach a method for increasing sialylation and/or N-glycan charge of a glycosylated protein. Furthermore, none of the cited references teach that sialylation is defined by N-glycan charge or that the sialylated protein comprises tri-, tetra- or pentasialo glycoforms of N-glycan.

Gawlitzek et al teach that in the culture of mammalian cells, the metabolite ammonium is produced as a by-product of glutamine metabolism and the thermal degradation of glutamine.

Gawlitzek et al further teaches that increased amounts of ammonium in cells leads to a decrease in terminal galactosylation and sialylation of TNFR-IgG. Thus, the reference provides a suggestion for increasing sialylation and/or N-glycan charge of a glycosylated protein in a cell without adding glutamine. Moreover, Gawlitzek et al teach that increasing the N-glycan of a glycosylated protein, i.e. increasing the ratio of glycosylation of a glycosylated protein, decides the activity of the protein.

Schneider et al teach that in the culture of mammalian cells, the metabolite ammonium is produced as a by-product of glutamine metabolism and the thermal degradation of glutamine. Schneider et al further teaches that increased amounts of ammonium in cells leads to a decrease in terminal galactosylation and sialylation of recombinant GCSF (see Table 1, page 163). Schneider et al further teach that it is a widely held view that the most important inhibitory substance accumulating in cell cultures is ammonia, and that a reduction in ammonia levels has to be overcome in order to achieve the successful economic scale-up of such processes for the production of high value proteins (page 165). According to this reference the main source of accumulating ammonia in mammalian cell cultures is amino acid metabolism, particularly that of glutamine. Glutamine has been shown to be the most major nutrient, in addition to glucose, for most growing animal cells in culture. Glutamine therefore serves as a protein constituent and as the main energy source (see page 165, 2nd col). At page 176, Schneider et al teach that one means of controlling excessive ammonia formation is to control the addition of glutamine in cells by limiting glutamine availability.

Thus, the reference sets forth a correlation between increased ammonia accumulation and decreased sialylation and/or glycosylation of a protein, and further suggests that limiting the

availability of glutamine in a cell would result in decreased ammonia accumulation. Page 176, 2nd ¶ of Schneider et al. teach the following:

"Bols et al. (1995) reported the successful growth of several fish cell lines in glutamine-free media. Fish cells, in contrast to mammalian cells, do not require glutamine. Thus they could be grown in media deficient of glutamine, glutamate and α-ketoglutarate without any adaptation. The observed differences with mammalian cells must be due to differences in the biochemistry of fish cells."

Hermentin et al teach a process for characterizing the glycosylation of glycoproteins based on a hypothetical charge number N. Hermentin et al teach that it is important to reliably determine the degree of glycosylation or sialylation in glycoproteins, such as erythropoietin, in order to gauge bioavailability/biological activity of a protein for therapeutic use. Hermentin et al discloses that when erythropoietin is incompletely glycosylated, it is quickly cleared from the blood circulation and would not be biologically useful (see column 1, lines 6-15, 28-45 and column 2, lines 14-25, for example). Hermentin et al teach that it is crucial to determine the distribution of glycan groups exhibiting differing degrees of sialylation to be able to index the bioavailability of a glycoprotein. Hermentin et al teach that the N charge of a glycoprotein is determined in part by determining the percentage of trisialo, tetrasialo and pentasialo ranges (see column 3, lines 27-50, column 4, lines 27-35, column 5, lines 4-12, for example). Hermentin et al teach that the N-glycan charge value was determined for EPO and erythropoietin is comprised of trisialylated N-glycans and tetrasialylated glycans (see column 12, lines 43-52, for example), which meets the limitation of EPO as a sialylated protein comprising tri and tetrasialylated glycoforms defined by N-glycan charge and a process of defining sialylation by N-glycan charge.

It would have been obvious to the skilled artisan at the time the invention was made to combine the cited references in the design of the instant invention. One of ordinary skill in the art would have been motivated to modify the method taught by Bebbington et al from making a rodent glutamine auxotrophic cell to produce an exogenous sialylated protein such as tPA to a human cell as taught by Brandt et al to produce a human exogenous sialylated protein because Brandt et al disclose that human cells are preferred for the preparation of human proteins in order to produce human proteins in economical yields in a form that is suitable for production of a pharmaceutical preparation. The motivation to make a human glutamine auxotrophic cell that is transfected with a glutamine synthetase sequence and a separate vector comprising a sequence for an exogenous sialylated protein is the expected benefit of being able to use the cells to synthesize a target protein with a sialic acid moiety glycosylation pattern that is comparable to that of the naturally occurring target protein (see column 3, lines 23-30, for example). Moreover, Wilson et al. expressly teach the exogenous expression of glutamine synthetase in glutamine auxotrophic cells.

It also would have been obvious to the skilled artisan at the time the invention was made to modify the method and cells taught by Bebbington et al and incorporate a second exogenous DNA sequence as a selection marker and amplification gene because Brandt et al teaches clone selection and gene amplification using a positive or negative selection marker. The motivation to use a selection marker and amplification gene such as DHFR to make a cell is the expected benefit as disclosed by Brandt et al of being able to use a gene with a sensitivity for a selection agent in order to increase the expression of an gene to be produced by culturing the cell in the presence of increasing concentrations of a selection agent (i.e. methotrexate) (see column 10,

lines 36-44, for example). There is a reasonable expectation of success to make a human glutamine auxotrophic cell wherein these exogenous DNA sequences are located on more than one DNA vector construct, because similar methods have worked previously in the references cited. Therefore, Bebbington et al in view of Brandt et al render obvious a method to produce the glutamine-auxotrophic human cell used in claim 32.

It also would have been obvious to the skilled artisan at the time the invention was made to modify the method taught by Bebbington et al and make a glutamine auxotrophic cell that is capable of growing in serum-free medium because Brandt teach the importance of serum-free medium for cultivation. The motivation to use a serum-free medium is the expected benefit of being able to reduce the danger of contamination of the protein produced with animal pathogens that might be introduced by using animal serum (see column 2, lines 22-35, for example). There is a reasonable expectation of success to make a human glutamine auxotrophic cell with these limitations, because similar method have worked previously in the references cited. Therefore Bebbington et al as evidenced by Barsomian et al in view of Brandt et al render obvious a method to produce the glutamine-auxotrophic human cell of (instant claim 32) wherein the cell is further adapted to growth in serum free medium as claimed in claim 36.

It would have been obvious to the skilled artisan at the time the invention was made to determine the N-glycan charge for the sialylated protein such as EPO or tPA being produced by the cell because Hermentin et al teach that it is important to know the degree of glycosylation of recombinant therapeutic proteins such as erythropoietin, since slightly altered glycosylation patterns can drastically effect the activity of the therapeutic protein. The motivation to determine the N-glycan charge is the expected benefit of being able to determine the degree of

glycosylation in a simple, reliable manner suitable for replacing the methods previously known in the art for determining the bioavailability of a therapeutic protein before use. There is reasonable expectation of success in combining the protein production method using a glutamine auxotrophic cell rendered obvious by Bebbington et al in view of Brandt et al with the methods taught by Hermentin et al and use a glutamine- auxotrophic human cell transfected with an exogenous DNA sequence encoding a glycoprotein, such as erythropoietin, to produce and recover erythropoietin and determine its bioavailability via N-glycan charge, because these methods have worked before in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore, Bebbington et al as evidenced by Barsomian in view of Brandt et al and further in view of Hermentin et al render obvious a cell and a process for the production of a sialylated protein comprising culturing a glutamine-auxotrophic human cell in glutamine free medium and recovering the expressed sialylated protein comprising tri-, or tetrasialo glycoforms of the glycan defined by an N-glycan charge.

Additionally, it would have been obvious to the ordinary skilled artisan at the time of the instant invention, to modify the teachings of Wilson et al. (WO 87/04462 A1) or Bebbington et al (U.S. Patent No. 5,891,693, of record) as evidenced by Barsomian et al (U.S. Patent No, 5,238,821, of record) in view of Brandt et al (U.S. Patent No. 6,395,484, of record) by the teachings of Schneider and Gawlitzek et al. The ordinary skilled artisan seeking to increase the sialylation and/or N-glycan charge of a glycosylated protein, would have been motivated to express the desired protein in a cell without adding glutamine, since the prior art clearly teaches

that ammonium, a byproduct of glutamine metabolism has a direct effect on the level of sialylation and/or N-glycan charge of a glycosylated protein.

Claim 32 recites in the preamble that the method is, *inter alia*, for "extending the viability of said cell", and a similar limitation is recited in the last line of the claim. In a general sense, the instant specification only discloses extending cell viability using media supplements (page 10, lines 15-25), not by use of the glutamine synthetase transgene. In a narrow sense, a single clone, 3E10, was found to have extended viability in Example 10. Although not specifically stated, this is implied to be due to the glutamine synthetase transgene. Claim 32 is not limited to any specific cell type or clone, thus, the instant specification relies as much upon the prior art for this limitation as does the above rejection because of the lack of any broad, general teaching that the addition of a glutamine synthetase transgene will provide the claimed increase in cell viability. The general concept and thrust of the invention as disclosed is towards the use of the glutamine synthetase transgene in transfected cells in order to mediate glycosylation of proteins, not towards the extension of cell viability. Furthermore, cell viability is extended in the instant claims as compared to what? The parent cell line, untransfected with glutamine synthetase? Any chosen cell line or cell type? Given the above and the teachings of the prior art regarding the benefits of using glutamine-free media and cells competent to grow in such media, it is considered that this limitation is met by the combination of the prior art references above. In particular, the teachings of Schneider et al regarding the inhibitory nature of ammonia (a by-product of glutamine metabolism) in cell culture necessarily leads to a conclusion that reducing ammonia levels, e.g. by removing glutamine from the cell culture, would relieve cells from this inhibition, thus extending viability.

(10) Response to Argument

Applicant's arguments filed 7/12/2010 have been fully considered but they are not persuasive.

Applicants essentially assert that: 1) the instant application presents unexpected results with respect to human glutamine auxotrophic cells that increase sialylation or N-glycan charge of expressed proteins when transfected with a glutamine synthetase gene; 2) the prior art cited above does not suggest, or provide a reasonable expectation of success, that the recited cells could grow in glutamine free media in order to increase sialylation, N-glycan charge, or cell viability; 3) the claimed invention is contrary to the disclosure of Gawlitzek et al, and offer a different interpretation of this reference; 4) applicants provide their summary of the teachings of the references cited above, and assert that none of the remaining references teach the conclusion of Gawlitzek et al, i.e. linking glutamine concentration to terminal galactosylation and sialylation; 5) the treatment of the intended use limitation "extending the viability of said cell" by the Examiner is inappropriate

Regarding 1), there is little that is unexpected about this assertion when the prior art above is applied. The nexus between ammonia levels and glycosylation is provided by Schneider and Gawlitzek et al, whereas the nexus between glutamine and ammonia levels in cell culture is also provided by these same references.

Regarding 2), this assertion is not supported by any reasoning or evidence, and is directly refuted by the extensive disclosure of the prior art documents above regarding growing cells in glutamine-free media, and the nexus between glutamine free media and increased sialylation and N-glycan charge. With this assertion, applicants appear to desire a 35 USC 102 level of

disclosure in the prior art, but this is not the proper standard or analysis for this 35 USC 103 rejection.

Regarding 3), this line of reasoning, and the citation of certain parts of Gawlitzek et al, is less than clear because the teachings of Gawlitzek et al directly contradict applicants assertions. Despite an exhaustive listing of references and citations in the introductory section of Gawlitzek et al linking ammonium concentration with detrimental effects in cell culture, applicants grasp a sentence fragment (page 641, first column) and insist this refutes all of the prior findings along with those of Gawlitzek et al. Read in light of the entire reference, this sentence fragment is taken out of context by applicants, as sentence fragments often are. The results applicants are referring to in this instance, i.e. Figs. 1 and 2, include cells that were shifted to a temperature of 31° C in order to prevent apoptosis (or cell death), see the ¶ linking the first and second columns, page 638. This is an alternative explanation regarding the cell viability results cited by applicants.

Applicants next insist that Gawlitzek et al teaches that eliminating glutamine will not effect terminal galactosylation and sialylation. A review of the entirety of Gawlitzek et al reveals no such statement, and, applicants cite a portion of Gawlitzek et al that appears to directly refute their assertion (taken from page 14 of the Appeal Brief):

> "The results presented here strongly suggest that ammonium inhibits galactosylation and sialylation of TNFR-IgG N-glycans by pH-regulated mechanisms."

It is unclear, and applicants do not explain, how these findings of Gawlitzek et al are expanded to reach the instant assertion or conclusion. The clear correlation taught in the prior art between glutamine levels and ammonium levels stands, and is not refuted by the actual mechanisms by

which high ammonium levels exert their effects, i.e. that they are pH-regulated. That the relevant enzymes responsible for protein sialylation and glycosylation are pH-regulated appears irrelevant in the end as long as the ultimate cause of their inhibition (i.e. high ammonium levels) was known, and means for correcting the cause of the inhibition were also known (i.e. reducing or eliminating glutamine). That Gawlitzek et al present various figures and graphs trying to explain the biochemical basis of how a low pH effects the relevant enzyme inactivity is stipulated. However, barring some reasonable explanation as to how this mitigates against the instant rejection rather than enforcing it, applicants assertions along this line are held unconvincing. The basic conclusions of Gawlitzek et al stand, and are summarized in the section on page 644, second column, provided by applicants. Gawlitzek et al appear to have eliminated certain biochemical explanations for the low pH - enzyme inhibition linkage (i.e. Figs. 6 - 7), but provide evidence that an increased pH (which inhibits both enzymes, Fig. 8) caused by ammonium in intracellular compartments is a plausible explanation (Table I, and accompanying text, page 643, second column to page 644). It is reiterated that in no sense does Gawlitzek et al teach or suggest what applicants are asserting: that reduction of ammonium levels by elimination of glutamine would not effect galactosylation and sialylation. Applicants present no reasoning regarding how they arrived at this conclusion.

Regarding 4), this assertion ignores the teachings of Schneider et al as set forth above. Furthermore, it ignores the very point of a 35 USC 103 rejection, i.e. that the rejection is based upon a combination of the references.

Regarding 5), this claim limitation has been addressed above. It is noted again the deficiencies of the instant application as applied to such a broad claim, and the basis for

comparison of "extended viability" remains far from clear. One interpretation of "extended viability" is that cells transfected with glutamine synthetase have "extended viability" as compared to cells that have already died in the cell culture. Another is that cells transfected with glutamine synthetase have "extended viability" compared to cells that have not been so transfected and grown in glutamine-free medium (an essential nutrient for certain cells, e.g. the teachings of Bebbington et al).

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

/Michael Burkhart/

Primary Examiner, Art Unit 1633

Conferees:

/Joseph T. Woitach/

Supervisory Patent Examiner, Art Unit 1633

/Peter Paras/

Supervisory Patent Examiner, Art Unit 1632